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Single-Use Sensor Strips for Reliable Field Analysis of Gunshot Residue



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14. ABSTRACT This report summarizes our activity over the past 24 months of the project titled 'Single-Use Sensor Strips for Reliable Field Analysis of Gunshot Residue'. Our team's accomplishments over the past two years include developments made on four major thrusts: (1) fabrication and assay optimization of the GSR sensor strip to demonstrate the simultaneous measurement of several GSR relevant heavy metals using a short assay time, (2) integrated sample protocol with the sensor strip towards efficient collection and negligible contamination, (3) advanced signal processing towards successful data reduction and feature extraction, along with the generation of unique GSR fingerprint patterns and (4) design of hand-held analyzer to demonstrate a complete prototype containing the required electronics, power systems, along with advanced signal processing.					
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Executive Summary

This report summarizes our activity over the past 24 months of the project titled ‘Single-Use Sensor Strips for Reliable Field Analysis of Gunshot Residue’. Our team’s accomplishments over the past two years include developments made on four major thrusts: (1) fabrication and assay optimization of the GSR sensor strip to demonstrate the simultaneous measurement of several GSR relevant heavy metals using a short assay time, (2) integrated sample protocol with the sensor strip towards efficient collection and negligible contamination, (3) advanced signal processing towards successful data reduction and feature extraction, along with the generation of unique GSR fingerprint patterns and (4) design of hand-held analyzer to demonstrate a complete prototype containing the required electronics, power systems, along with advanced signal processing.

The protocols implemented to successfully demonstrate these accomplishments include, but are not limited to development of single-use micro-fabricated electrode sensor strip for highly reliable, fast and simplified field detection of GSR. This yielded a user-independent sensor similar to glucose meter containing all required electronics, power systems and signal processing which allowed us to maximize available information and minimize false results. In the course of this work we generated distinct electrochemical GSR signatures using ‘green electrode’ strips (no mercury) using electrochemical stripping voltammetry to create unique signal fingerprint to eliminate false positives. We combined information-enhanced electrochemical analysis with powerful processing to obtain effective and rapid screening between subjects with different levels of exposure to GSR. Finally we validated this new system in connection to different types of weapons and ammunition in different control scenarios

Such productive efforts have resulted in 6 publications in leading international journals, and one provisional patent, reflecting the high scientific impact of our work. Our effort has also involved the training and development of one undergraduate student, 1 graduate student, and 2 postdoctoral fellows providing them a unique experience in the exciting and interdisciplinary research environment, developing their scientific excitement, and exposing them early in their career to an advanced stimulating research environment.

Problem/background

Reliable field analysis of gunshot residues (GSR) is extremely important for variety of battlefield scenarios, anti-terrorist applications and forensic investigations, in general. Such immediate and correct identification of individuals firing a weapon in the battlefield represents a very challenging task. The goal of this activity has been to develop and test single-use micro-fabricated electrode sensor strips, along with a held-held meter, for highly reliable, fast and simplified field detection of heavy metals from gunshot residues (GSR). Electrochemical stripping voltammetry represents a powerful tool for decentralized metal testing that can meet the demands of field GSR detection. Micro-fabricated (thick-film) sensor strips integrated the sample collection step with the generation of distinct electrochemical GSR 'signatures' (involving inorganic metal constituents such as Sb, Pb or Ba), towards minimization of contamination and false results. The PI pioneered similar disposable heavy-metal detection strips for on-site environmental testing and clinical screening of blood lead, as well as 'Green' carbon strips (that replace traditional mercury electrodes). The simultaneous measurements of the metal GSR with common organic constituents (propellants) have been evaluated to further enhance the information content.

Among the metallic constituents of such residues, antimony (Sb), lead (Pb) and barium (Ba) are nearly universally found in relatively high levels on the handler of a firearm subsequent to a discharge. Such inorganic gunshot residues are commonly analyzed using large metal analyzers based on graphite-furnace atomic absorption spectroscopy or inductively coupled plasma atomic emission spectrometry. However, these techniques are costly and bulky with respect to equipment, and require specialized personnel. Apart from this factor, the detection of these species alone can be inconclusive in criminal investigations since minimal amounts of these are not unique to GSR. Antimony is found in several alloys and oxides for fire retardants, lead is found in plumbing materials, solder and glass, and barium is found in paint and auto grease. In fact, workers in auto mechanic, electricity and construction industries were found to have higher levels of these metals on their hands. Combining the analysis of these heavy metals with the generation of distinct electrochemical fingerprints has further enhanced the reliability of GSR forensic investigations.

Sample collection is a crucial step in the analysis of GSR. Several dry and wet approaches are commonly employed for the collection of primer-metal residues such as tape lifts, vacuum lifts and swabbing, but require centralized facilities to proceed and are thus often confined to a laboratory. However, in this work the integration of sampling and analysis through abrasive stripping voltammetry (AbrSV) obviated the need for further sample treatment and thus rendered the device field-deployable.

The user-independent screen-printing sensor was integrated with a hand-held battery-powered electrochemical meter (similar to pocket-size glucose meters used by diabetics) to offer rapid and reliable GSR assays upon collecting the sample. Such a small meter contained all required electronics, power systems, along with advanced signal processing. Such signal

processing algorithms were developed towards maximizing the available information while minimizing false results for making an optimum 'Yes/No' decision regarding the suspect identification. The user-friendly software will control the entire operation, and coupled with the simplified protocol, minimized the need for operator training, as desired for field use by military operators with limited forensic training. Increased evidence values were achieved via simultaneous measurement of metal residues with organic (propellant) constituents. Flexible thick-film electrodes based on different materials were investigated to maximize the number of metals determined in a single GSR assay. Wearable textile-based printed electrodes were also examined towards a 'Lab-on-Sleeve' forensic field analysis. New protocols for integrating the sample collection with the sensor strip were developed, including placing of the adhesive tape collector directly onto the flexible electrode area, using the planar electrode to mechanically transfer the residue to the surface.

Methodology to solve

The goal of this activity was to develop easy-to-use micro-fabricated electrode strips for reliable and rapid field detection of heavy metals from analysis of GSR. Anodic stripping voltammetry (ASV) is a well-established trace-metal electroanalytical technique, recognized as a promising tool for the analysis of GSR. Its remarkable sensitivity and sub-ppb detection limits are attributed to the 'built-in' preconcentration step, during which multiple target metals are accumulated (electrodeposited) onto the working electrode. Following the deposition period an anodic potential scan is initiated, during which the metals are re-oxidized and 'stripped' away from the surface and the multi-peak voltammogram is recorded. The metal concentrations are deduced by the magnitude of the current signals (peak currents) arising from this stripping step. Up to 5-6 metals can be detected in a single run. The identity of each metal can be inferred from the peak position (potential) through spectral analysis. The compact (portable) instrumentation and low power demands of stripping analysis, along with its low cost and simplicity, make it particularly attractive for meeting the requirements for decentralized field GSR measurements. However, early applications of electrochemical stripping analysis of GSR involved mercury electrodes and relied on bench-top laboratory-based instrumentation along with bulky user-dependent 'beaker-type' cells. Replacing these mercury electrodes and cells with non-toxic disposable strips, in connection to a greatly simplified (user-independent) operation - analogous to that used by diabetes for self-testing of blood glucose - will be particularly useful for battlefield testing by relatively untrained soldiers.

The proposed activity was aimed at developing easy-to-use "one-shot" sensor strips for detecting GSR based on new 'green' carbon electrodes introduced by the PI's team. Such high-performance 'mercury-free' carbon electrodes offer a well-defined and highly reproducible stripping response, with high signal-to-background characteristics and resolution, comparable to those of common mercury electrodes. Single-use sensor strips – analogous to disposable glucose biosensors - eliminated cross contamination and carry-over problems (common to conventional re-usable electrodes) and obviated the need for surface polishing/renewal, solution replacement, or cell cleaning. The use of 'green' carbon-based disposable strips also addressed environmental restrictions of using and disposing toxic mercury electrodes and will hence reduced related hazardous waste disposal costs.

The new micro-fabricated (thick-film) sensor strips integrated the sample collection step with the generation of electrochemical 'signatures' for GSR based on distinct signals of its primer elements Sb, Pb and Cu. Such 'green' carbon microelectrode addressed environmental concerns related to the use and disposal of mercury-based sensors. Further minimization of contamination and analyte loss, and hence of false results, were achieved by integrating the sampling and measurement steps. Such quantization was coupled with an advanced signal processing that facilitated the correct decision regarding the suspect identification. Various sampling protocols were evaluated and compared for such direct integration with the micro-fabricated sensing element. These included placing the adhesive collection tape directly onto the strip, mechanically transferring the GSR onto a flexible electrode, or direct transfer of nitric-acid treated sampling

swab. The integrated GSR sensor strip underwent systematic optimization, detailed characterization and critical testing.

In the first part of this report, we addressed Task 1 of the proposal for this work: **Fabrication and assay optimization**. Here we describe for the first time the ability of electrochemical techniques to measure simultaneously – in a single voltammetric run – both heavy-metal and organic propellant constituents of GSR. In particular, we illustrated the ability of cyclic square-wave voltammetric route for obtaining such well-defined qualitative signatures for trace levels of targets, with enhanced information content compared to analogous cyclic voltammograms. Such a unique and powerful electrochemical route is based on the well defined redox behavior of nitro-containing explosives and propellants and on the sensitive stripping voltammetric detection of heavy metals.

In the second part of this report, we addressed Task 2: **Integrated sample collection**. Here we describe a novel “swipe and scan” protocol which integrated the GSR sampling from the hands of subjects discharging a firearm with voltammetric detection using a screen printed electrochemical sensor. Such integrated sampling/detection addressed the key challenge of instant identification of a subject who has discharged a firearm, as well as the preservation of the sample integrity (i.e., minimizing errors associated with the transport and storage of samples). Sampling and analysis were implemented using a novel protocol known as abrasive stripping voltammetry (AbrSV) based on an initial mechanical transfer of trace amounts of the GSR from the hand of the suspect directly to the surface of the working-electrode sensor strip without intermediate processing steps.

In the third part of this report, we addressed Task 3: **Advanced signal processing**. In this section we demonstrated a promising new method for assessing the levels of contact with GSR from the hands of different subjects using the integration of electrochemical signals with advanced chemometric data treatment. Our goal was to examine and maximize variations in the signals generated by multiple organic and inorganic GSR constituents from subjects who have discharged a firearm compared with those who have had secondary contact with GSR (in the presence of other shooters or contact with GSR contaminated surfaces). The work combined such information-enhanced electrochemical analysis and powerful CVA data processing with the goal of obtaining effective and rapid discrimination between subjects who have discharged a firearm and those who have not under a variety of relevant control scenarios. CVA analysis was thus used to classify samples from each control set examined. However, voltammetric/CVA analysis described here could not be incorporated into a hand-held device preventing portability and speed in forensic applications. Therefore it was subsequently replaced with a simpler model which examined metallic components of GSR only over a smaller electrochemical window based on Bayesian analysis. Bayesian analysis in this work was based on the idea that if you know the probability of detecting gunshot residue in a sample versus not detecting gunshot residue in a sample and you're given an unknown sample, you can compare the unknown with the known and determine if there is gunshot residue or not. In a simple, two class case (No GSR, GSR); it became a simple thresholding problem, where if the unknown sample has a higher concentration

of specific elements than the predetermined threshold of concentrations, we determined the sample contains GSR. The threshold was determined through a training set of data that was collected through controlled shooting range trips. From the training set, we extracted Gaussian parameters that allowed us to determine our threshold values. Specifically, we found the areas where GSR contains a high concentration of Cu and Pb and created two threshold values that separated GSR samples from non-GSR samples. This method was more suitable to real field-collected GSR samples acquired during field trips.

In the fourth part of this report, we addressed Task 4: **Design of hand-held analyzer**. In this section, we demonstrated a bread boarded prototype containing the required electronics, power systems, along with advanced signal processing. This system emulated a conventional analytical laboratory within a single device by leveraging prior expertise in metal analyzers. From field samples, Gaussian parameters were extracted to build detection model where Bayes decision rule was implemented on Arduino microcontroller. Old field samples were run with determined decision rule parameters and decision function tested further by scanning samples taken from used bullet cases. We established threshold levels between control scenarios of ‘Involved’ (load and fire) and ‘Not Involved’ (no contact, secondary contact with GSR and presence during discharge of a firearm). We designed set of ‘synthetic’ blind samples which were examined by device operator, without any prior knowledge of concentrations of each sample. All Contact samples were above threshold levels and correctly classified. Based on field tests, schematic and board layout for the device were finalized and the prototype was ordered and received.

In the fifth part of this report, we addressed Task 5: **Critical testing and validation**. The prototypes received from the manufacturers presented several challenges. Two such challenges involved the position of the LEDs and the operation of the linear regulator. These issues were addressed and solved. Further challenges with the device have been identified and may be addressed in future work of this project.

Results & Discussion

a. Task 1 - Fabrication and assay optimization

The simultaneous electrochemical measurement of heavy-metal and organic propellants relevant to gunshot residues (GSRs) was demonstrated. Cyclic voltammetry (CV) and cyclic square-wave stripping voltammetry (C-SWV) were shown to detect, in a single run, common propellants, such as nitroglycerin (NG) and dinitrotoluene (DNT), along with the heavy metal constituents of GSR, antimony (Sb), lead (Pb), zinc (Zn) and barium (Ba). The voltammetric

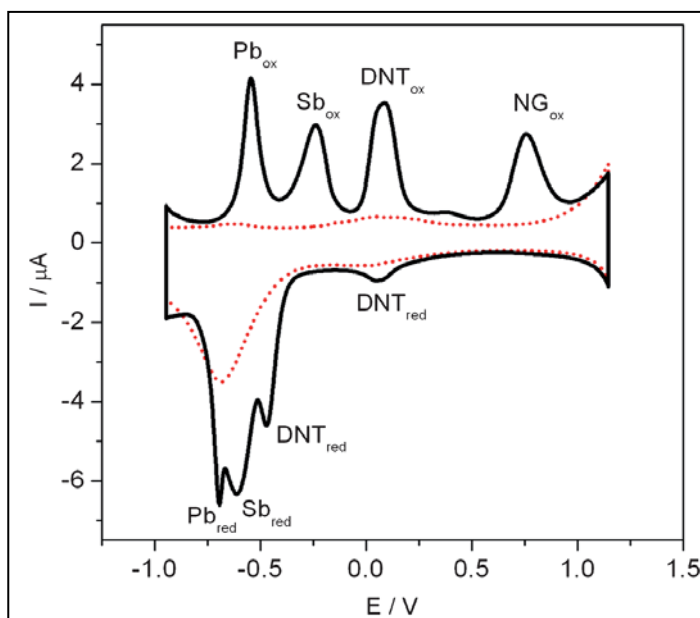
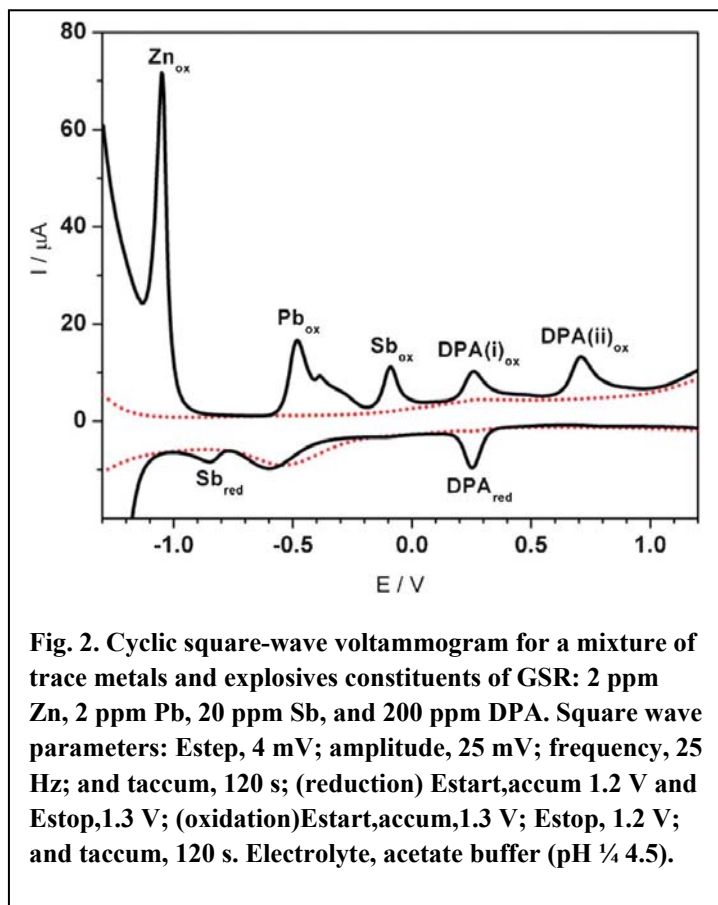


Fig. 1. Cyclic square-wave voltammogram at the bare GCE for a mixture of trace metals and explosives constituents of GSR: 3 ppm Pb, 10 ppm Sb, 50 ppm NG, and 10 ppm DNT. Square wave parameters: Estep, 4 mV; amplitude, 25 mV; frequency, 8 Hz; and teq, 5 s; (reduction) Estart, 1.15 V; and Estop, 0.95 V; (oxidation) Estart,accum, 0.95 V; Estop, 1.15 V; and taccum, 120 s. Electrolyte, acetate buffer (pH ¼ 4.5).

detection of the stabilizer diphenylamine (DPA) along with inorganic constituents was also examined. The resulting electrochemical signatures combined – in a single voltammogram – the response for the various metals and organic species, based on the reduction and oxidation peaks of the constituents. Cyclic square-wave voltammetry at the glassy carbon electrode (GCE), involving an intermittent accumulation at the reversal potentials of -0.95 V (for Sb, Pb, DNT and NG) and -1.3 V (for Sb, Pb, Zn and DPA) was particularly useful to offer distinct electrochemical signatures for these constituents of GSR mixtures, compared to analogous cyclic voltammetric measurements. Simultaneous voltammetric measurements of barium (at thin-film Hg GCE) and DNT (at bare GCE) were also demonstrated in connection to intermittent accumulation at the reversal potential of -2.4 V. Such generation of

unique, single-run, information-rich inorganic/organic electrochemical fingerprints holds considerable promise for ‘on-the-spot’ field identification of individuals firing a weapon, as desired for diverse forensic investigations. The research results on the new cyclic-square wave protocol were also summarized in the paper ‘Simultaneous electrochemical measurement of metal and organic propellant constituents of gunshot residues.’ *Analyst* **2012**, 137, 3265.

Fig. 1 displays a typical C-SWV at a bare glassy-carbon electrode (GCE) for a mixture of 4 common GSR constituents, the 2 heavy metal ions Sb^{3+} and Pb^{2+} and the 2 propellants NG and



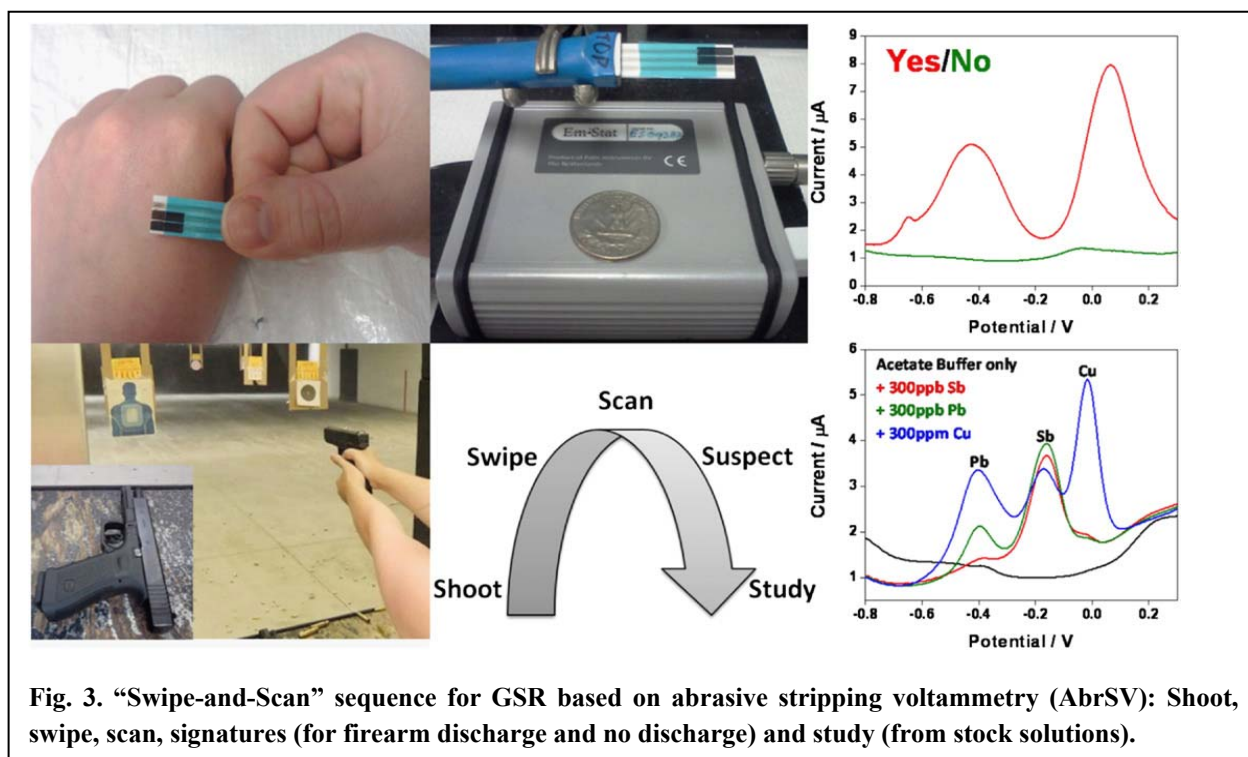
of these heavy metals and common propellants can lead to a distinct electrochemical signature, particularly in connection to cyclic square-wave voltammetry. We expect that such a unique, information-rich, single-run metal/propellant fingerprint will provide complete patterns of questioned and known signatures to determine if the constituents of the residue detected match those of suspects. We hope to implement the detection of all the species outlined in this work in a single, broad-potential scan at sensor-strip electrodes, utilizing both anodic and cathodic signals, to provide a portable GSR detection system.

b. Task 2: Integrated sample collection

Increasing security needs require field-deployable, on-the-spot detection tools for the rapid and reliable identification of gunshot residue (GSR), and thus the collection of GSR samples is a crucial step in forensic analysis. In this work we demonstrated a novel protocol integrating GSR sampling and electroanalysis using micro-fabricated carbon sensor-strips. The new integrated sampling/detection methodology relied on abrasive stripping voltammetry (AbrSV) involving an initial mechanical transfer of trace amounts of surface-confined GSR from the hand of a suspect directly onto the electrode contingent of the sensor strip, which was immediately ready for electrochemical analysis. The integrated sampling/detection method displayed much promise as a portable, rapid and inexpensive system to promptly identify a subject who has discharged a

DNT. A SWV was first swept from 1.15 V to -0.95 V reducing the explosives and metal ions. The potential was held at -0.95 V for 120 s and a SWV was swept back to 1.15 V, oxidizing and stripping the reduced products. Such a cyclic operation resulted in a well-defined voltammogram with distinct peaks of the metal/ metal ion and propellant species. The electrochemical detection of DPA, an organic stabilizer present in GSR, was examined at a bare GCE. As previously mentioned, studies on the constituents of smokeless gunpowder showed DPA to be present in at least 80% of the powders examined. Fig. 2 displays the electrochemical signals for DPA, Zn, Pb and Sb at a bare GCE.

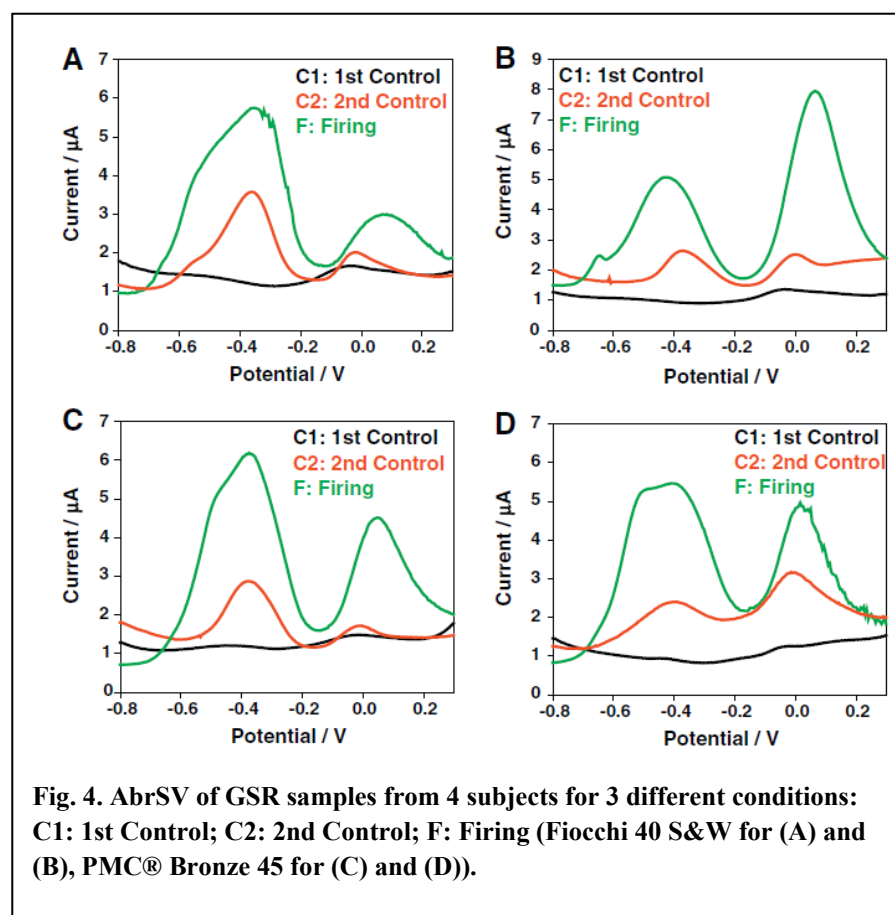
Our study illustrated that a mixture



firearm in various forensic scenarios. Such effort resulted in the publication of “Swipe and Scan”: Integration of sampling and analysis of gunshot metal residues at screen-printed electrodes. *Electrochemistry Communications* **2012**, 23, 52.

Fig. 3 outlines the sequence of the new “swipe-and-scan” technique. The subject discharged the firearm (bottom-left — “Shoot”). The sensor strip was rubbed over the subjects hand (top-left — “Swipe”). Buffer solution was dropped directly onto the electrode and ASV is implemented (top-middle — “Scan”). The output displays the results obtained without exposure to GSR (No) and post-firearm discharge (Yes) (top-right — “Suspect”). In the case of Yes, two voltammetric signals were observed. The signal at -400 mV vs. Ag/AgCl is attributed to Pb and the signal at 0 mV vs. Ag/AgCl is attributed mainly to Cu, but with contributions from Sb. This signature was compared to standard additions of Pb, Sb and Cu examined at the Au-CSPE in buffer (bottom-right—“Study”), and yielded a signal for Pb at -400 mV, a signal for Sb at -50 mV and a signal for Cu at 0 mV vs. Ag/AgCl. A clear signal was not observed for Sb in “Suspect” since levels of Sb in GSR are known to be significantly lower than both Pb and Cu; however, contributions from Sb were indicated by the shift of the Cu peak potential to a more positive value.

The effect of the “swipe-and-scan” technique for the detection of GSR before and after discharging a firearm was investigated. Fig. 4 illustrates the results obtained from 4 different subjects under the following conditions: C1: 1st Control; C2: 2nd Control; F: Firing. C1: 1st Control voltammetry (black) displays a small signal at a potential of -40 mV vs. Ag/AgCl, which is attributed to trace levels of Cu. C2: 2nd Control voltammetry (red) shows two signals at -400 mV and 0 mV vs. Ag/AgCl, which are attributed mainly to Pb and Cu/Sb contributions, respectively. The Pb signal was not present prior to exposure to GSR but substantially increased



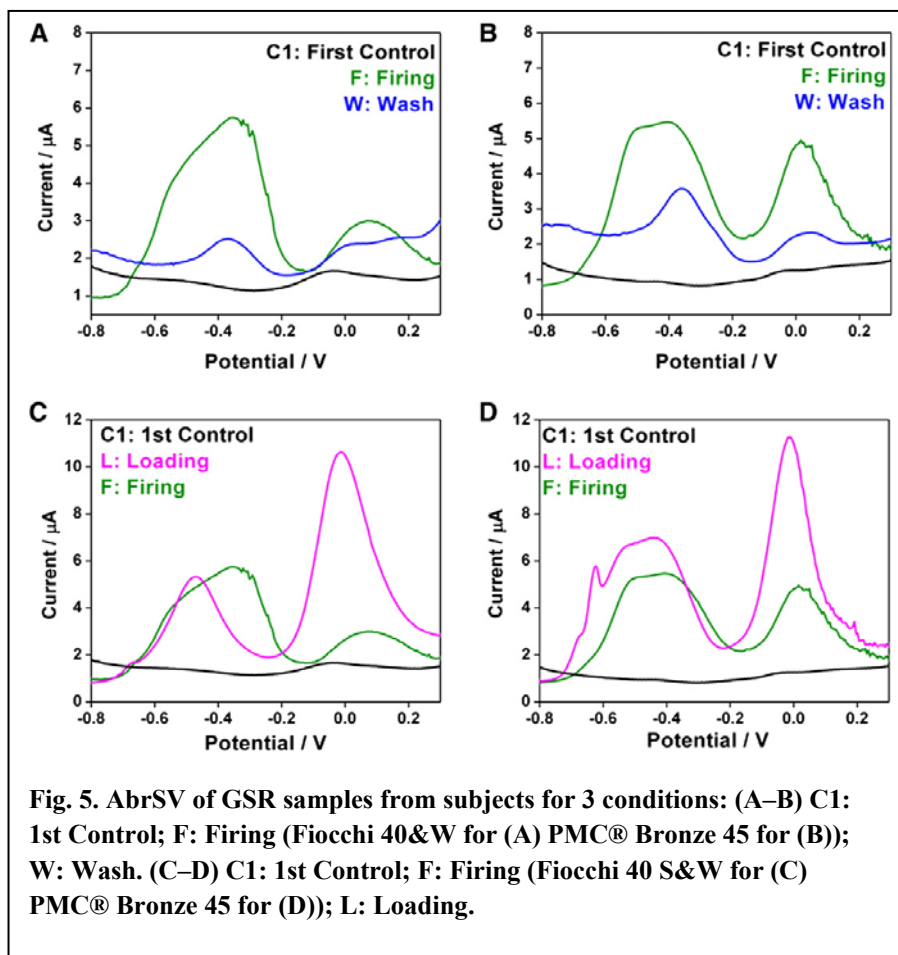
of Pb at -400 mV vs. Ag/AgCl, and the peak corresponding to Cu/Sb at 0 mV. Another signal was observed at -600 mV which may be attributed to the stripping of a Pb/ Cu or Pb/Sb amalgam, as it increased with higher Sb/Cu signals. Therefore, it is clear that the level of GSR on the hands of a shooter greatly exceeds that on the hands of an observer.

The voltammetric effect of washing a subject's hand post-discharge of a firearm was investigated using AbrSV. Fig. 5(A–B) outlines voltammetric signatures of subjects who have discharged a firearm shown in green — F: Firing, samples taken post-hand washing in blue (W: Wash), and C1: 1st Control (black). The hand wash test (W: Wash) showed a decrease in the Pb and Cu signals at -400 mV and 0 mV vs. Ag/AgCl, respectively. However, the magnitude of the current for both these signals was still greater than those observed prior to the subjects' contact with a firearm, C1: 1st Control. This substantiates that significant effort must be made to remove traces of GSR from the hands after exposure to GSR or the discharge of the firearm.

The effect of AbrSV was also examined on the hands of subjects who loaded the firearm, compared with voltammetry after discharge. Fig. 5(C–D) outlines the voltammetric signatures for AbrSV of subjects who fired weapons, F: Firing (green). The results were compared with C1: 1st Control (black) and AbrSV samples taken from the hands of the corresponding subject subsequent to loading the firearm, labeled L: Loading (magenta). A significant increase in the level of Cu for the L: Loading-scans was observed, compared to the corresponding discharge of

thereafter. The levels of Cu and Sb also became elevated when compared to C1. The increase in these signals is consistent over 4 different subjects, despite no steps taken to moderate the length of time and proximity to the discharge of firearms. This signature demonstrates that GSR can travel not only to the shooter, but also to observers in their vicinity. Finally, the signature from a strip taken post-discharge of a firearm, F: Firing (green) showed a significant increase in the electrochemical signals

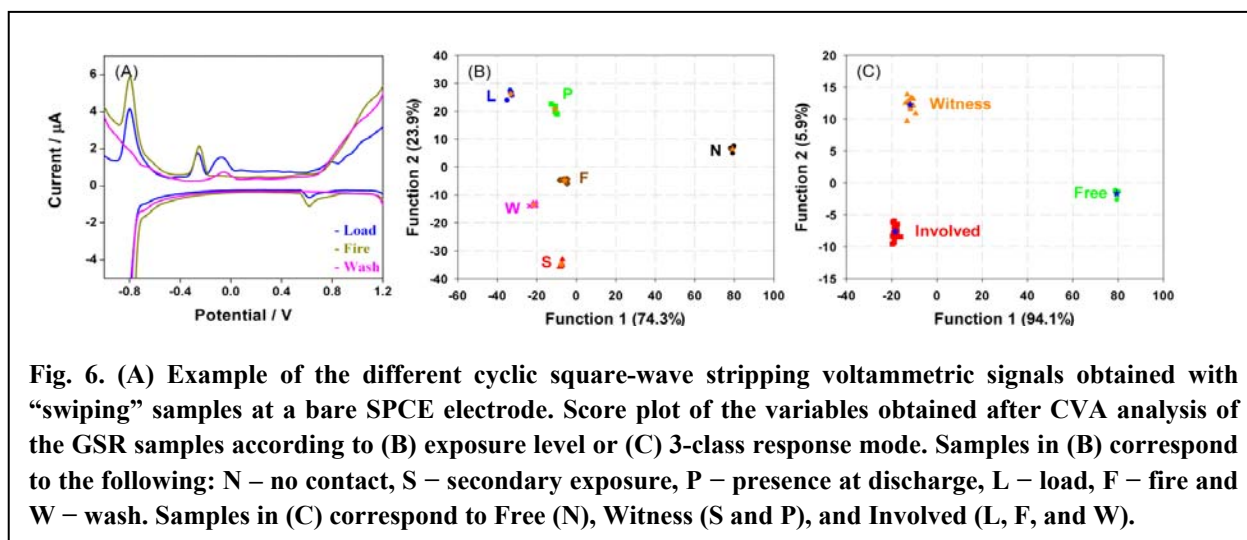
the firearm. This may be due to increased exposure to Cu from the brass bullet casing. The levels of Pb were also comparable with those detected upon discharge of the firearm, whereby the overall signatures of GSR are similar, suggesting that high levels of GSR remain on the firearm from previous discharge. The similarity in these signals as well as the prevalence of these metals was noted in several industries such as automotive, so that further refinement is required to minimize false positives.



The new AbrSV protocol was based on an initial mechanical transfer of trace amounts of the immobilized GSR from the hand of the suspect directly to the working electrode surface of the sensor strip obviating the need for intermediate processing steps. The use of this fast and inexpensive protocol has been found to be effective for immediate analysis using ASV. Future refinements to this work will involve inclusion of organic species present in GSR in connection to wider (anodic) potential windows. We propose that this sample-collection method shows much promise for a portable, field-deployable system aimed at rapidly identifying a subject who has loaded or discharged a firearm.

c. Task 3: Advanced signal processing

We demonstrated a novel system for the detection and discrimination of varying levels of exposure to gunshot residue from subjects in various control scenarios. Our aim was to address the key challenge of minimizing the false positive identification of individuals suspected of discharging a firearm. The chemometric treatment of voltammetric data from different controls using Canonical Variate Analysis (CVA) provided several distinct clusters for several scenario examined. Multiple samples were taken from subjects in controlled tests such as secondary



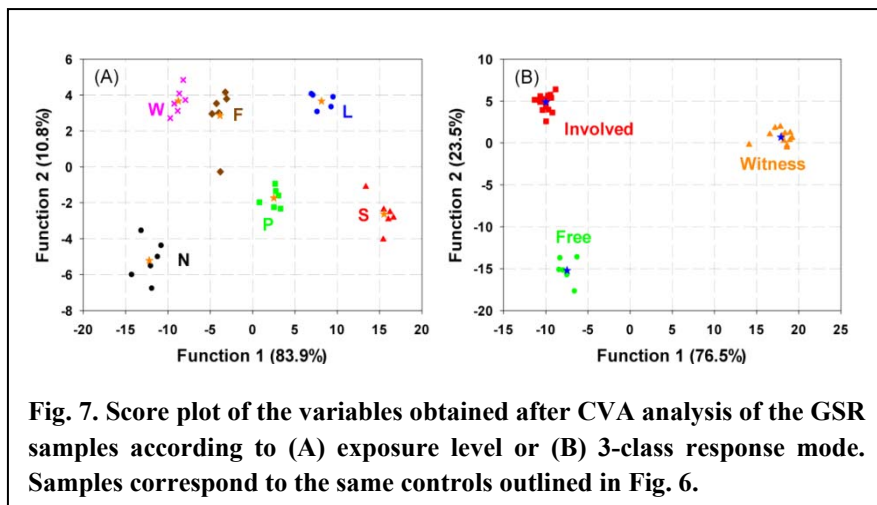
contact with gunshot residue (GSR), loading a firearm, and post discharge of a firearm. These controls were examined at both bare carbon and gold-modified screen-printed electrodes using different sampling methods: the ‘swipe’ method with integrated sampling and electroanalysis and a more traditional acid-assisted q-tip swabbing method. The electroanalytical fingerprint of each sample was examined using square wave voltammetry; the resulting data were preprocessed with Fast Fourier Transform (FFT), followed by CVA treatment. High levels of discrimination were thus achieved in each case over 3 classes of samples (reflecting different levels of involvement), achieving maximum accuracy, sensitivity, and specificity values of 100% employing the leave-one-out validation method. This system may serve as a potential method for a portable, field-deployable system aimed at rapidly identifying a subject who has loaded or discharged a firearm to verify involvement in a crime, hence providing law enforcement personnel with an invaluable forensic tool in the field. Such effort has resulted in the publication of ‘Rapid Field Identification of Subjects Involved in Firearm-Related Crimes Based on Electroanalysis Coupled with Advanced Chemometric Data Treatment’. *Analytical Chemistry* **2012**, *84*, 10306.

Fig. 6A displays an example of some of the voltammetric signals resulting from the samples taken from subjects at the gun range using the firearms and ammunition described in Firearms and Ammunition. The voltammetry from samples for L-Load, F-Fire, and W-Wash are shown. Acetate buffer was dispensed onto the electrode surface subsequent to sampling, and SWV was carried out in the potential range of -1.3 V to $+1.3$ V vs. Ag/AgCl (outlined in Square-Wave Voltammetry). The oxidative voltammetry exhibited 3 stripping signals at potentials -0.8 V, -0.275 V, and 0 V vs. Ag/AgCl. These were attributed to anodic stripping of metals, possibly zinc + nickel amalgams, lead, and copper, respectively. The signals for lead and copper and for nickel–zinc alloys have already been observed at similar potentials at a glassy carbon electrode (GCE). Each of these species was noted to be present in the ammunition according to the material safety data sheets. The signal at 0 V (vs. Ag/AgCl) was much greater for the L-Load scan than it is for any of the other scans. This signal was attributed to Cu, and the increase in the

signal for the L-scan was attributed to increased contact of Cu from the brass bullet case. This electrochemical behavior was previously observed by the authors using the 'swipe' method of GSR collection. Further signals were observed at more positive potentials on the cathodic sweep, which were attributed to the metallic components of GSR. The use of this 'cyclic' SWV waveform to deliver such a detailed electroanalytical fingerprint, in a single voltammetric run, demonstrated the intrinsic advantages of simplicity and rapidity of this method for the detection of the components of GSR.

Clear discrimination of the samples was achieved at these electrodes upon CVA treatment of this data, as seen in Fig. 6B, with patterns in the figure evidencing that samples are grouped according to the exposition level, where these well established clusters clearly separate the 6 classes of samples. As expected from the CVA plot, nearly all samples were correctly classified according to exposure level. Hence, the classification rate of the samples reached an accuracy of 86.1%. The efficiency of the classification obtained was also evaluated according to its sensitivity, i.e. the percentage of objects of each class identified by the classifier model, and to its specificity, the percentage of objects from different classes correctly rejected by the classifier model. The value of sensitivity, averaged for the classes considered, was 86.1%, and that of specificity was 97.2%. This method provided clear discrimination of various control scenarios for a number of subjects over a very short time scale with facile sampling and analysis. As mentioned above, CVA was also performed on samples taken using the swiping method from the back of the hand only and resulted in a classification rate 66.7%, sensitivity of 66.7%, and specificity of 93.3%.

To improve model reliability and to provide a simpler response outlining a subject's complicity, classification of samples was also attempted shrinking previous data to a 3- class study case. As per Fig. 6B, a new CVA model was built and evaluated using the leave-one-out cross-validation method. In this case, only three groups were considered, Free, Witness, and Involved, and the CVA model was formed by just two canonical variables (CVs). The same data used for Fig. 6B was also used in this model, whereby Free encompasses N-No contact, Witness uses the previous data for S-Secondary contact and P-Presence at discharge, and Involved uses the data for L-Load, F-Fire, and W-Wash. Fig. 6C outlines the clear discrimination for the samples based on the three exposure level categories. Patterns in the figure evidenced that samples are grouped according to degree of involvement. According to this classification, the Free cluster was still far removed from the other two clusters, which had similar merits for CV 1 but that were clearly separated by CV 2. Similar behavior was observed for Witness and Involved, whereby no overlap with the other categories was observed and clear discrimination along the axes was obtained, providing a simple system for the discrimination of subjects in different scenarios based on their exposure to GSR. Furthermore, as with the previous case for 6 categories (Fig. 6B), a confusion matrix was constructed employing a leave-one-out cross-validation approach. As expected from the CVA plot, all samples were correctly classified, with a classification rate of 100% in terms of accuracy. Also the same values for sensitivity and specificity were obtained for the method. An interesting noticeable feature was the reduced



GSR samples from a subject suspected of discharging a firearm. As per SPCE Swipe, a new CVA model was constructed to assess the discrimination capabilities when swabbing was utilized as the method to obtain GSR samples from a suspect. These samples were also measured at bare SPCEs. The samples taken for different control scenarios are the same as those outlined in SPCE Swipe (resulting in 35 samples; due to error, 1 sample was lost). Fig. 7A shows the score plot of the first two canonical variables obtained after CVA analysis of GSR samples according to 6 classes (observed in Fig. 6B), while Fig. 7B displays the classification of swabbing samples according to a simpler response mode (observed in Fig. 6C). We observed larger dispersion along cluster centroids in both Figs 7A and 7B, compared to the ‘swiping’ procedure of Fig. 6, although discrimination for all the classes was still achieved. Examining the obtained scores plot in Fig. 7A, we observed that N-No contact samples appeared further from the other classes but not by as much as in previous cases using the ‘swiping’ method of sample collection. That is, N-No contact samples had lower CV 2 score values; P-Presence and S-Secondary contact samples had similar intermediate CV 2 score values, being discriminated by CV 1; and W-Wash, F-Fire, and L-Load samples presented the same behavior. An analogous model was obtained for the simpler approach (previously observed in Fig. 6C) but with clearly less dispersion along the centroids; i.e. groups sorted according to exposure level along CV 2, Free group further from the others, and Involved and Witness groups mainly separated by CV 1.

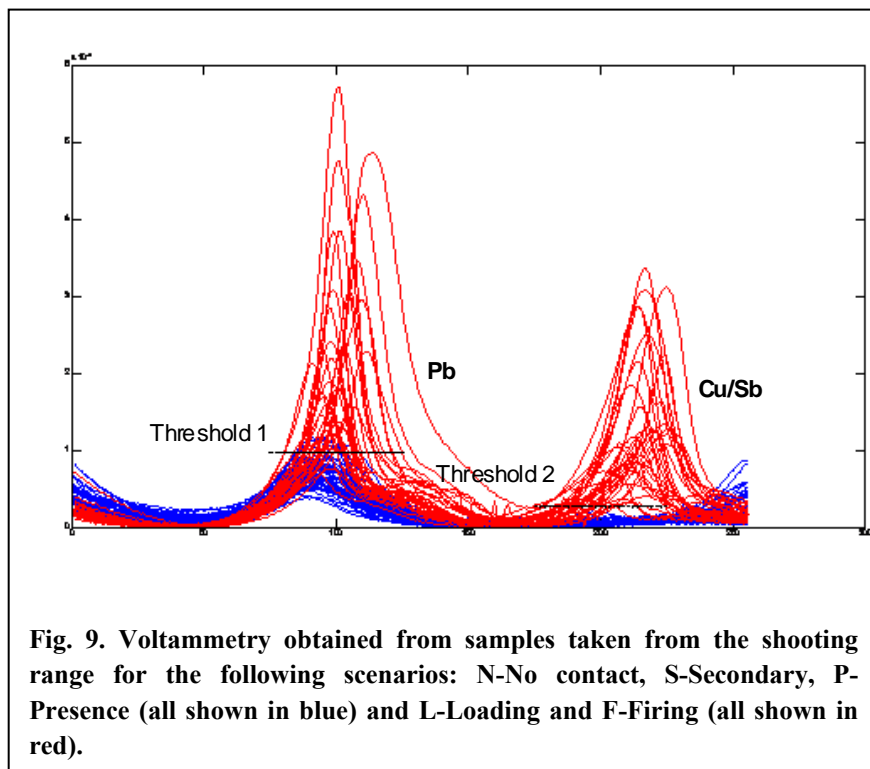
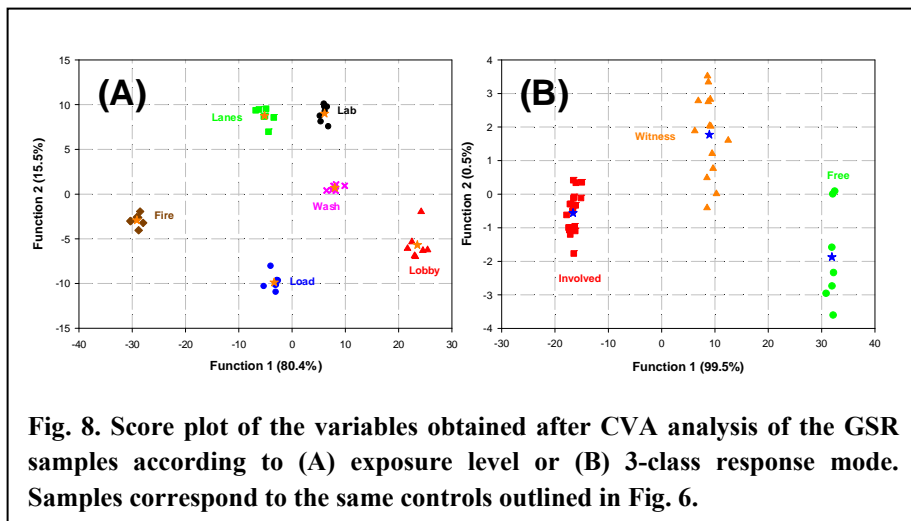
dispersion on the attained grouping, especially in contrast with the high separation between groups; this feature may constitute highly reliable decision systems, where ‘no doubt’ cases appear.

Swabbing assisted with diluted acid is traditionally used in electroanalysis to obtain

We proceeded to use a similar model to Fig. 6 to validate the system on blind samples taken from the gun range. Firstly, we printed a batch of carbon screen printed electrodes. We utilized 36 samples for the 6 control scenarios outlined in Fig. 6B to develop a model, the

results of which are shown in Fig. 8A. Tight clustering was observed with an accuracy of >95% for the training samples. We validated this using the same electrode batch, but taking samples on a different day. Unfortunately, the majority of samples were misclassified yielding an accuracy of 40% for the blind samples. Most of the misclassification was between similar control scenarios. For example, N, S and P were misclassified within each other's categories, as were L and F. We therefore decided to repeat this exercise utilizing the simpler system detailed in Figs 6C and 7B. The model was built with almost 100% accuracy, shown in Fig. 8B, and validated with 70% accuracy, a significant improvement on before. However, upon further inspection, given the technology available to us, this chemometric technique was not feasible in a small, portable, hand-held device. We therefore reexamined the voltammetry from different scenarios to come up with the best alternative for the system.

The voltammetry in the potential range of -1 V to 0.1 V for different scenarios for samples of GSR are shown in Fig. 9. The samples shown in blue are designated as 'Not Involved' and include the scenarios of N-No contact, S-Secondary contact, and P-Presence for discharge. The samples shown in red



are designated as ‘Involved’ and include the scenarios of L-Load and F-Fire. We observed a significant difference in the voltammetric fingerprint for each scenario. We therefore decided to develop more of a screening tool for use by minimally trained operators in the field, to give a high-speed/high-fidelity response as to whether someone had loaded/discharged a firearm or not. This was conducted using Bayesian analysis. Bayesian analysis in this work is based on the idea that if you know the probability of detecting gunshot residue in a sample versus not detecting gunshot residue in a sample and you’re given an unknown sample, you can compare the unknown with the known and determine if there is gunshot residue or not. In a simple, two class case (Not Involved, Involved), it became a simple thresholding problem, where if the unknown sample had a higher concentration of specific elements than the predetermined threshold of concentrations, we determined that the sample contained GSR. The threshold was determined through a training set of data that was collected through controlled shooting range trips. From the training set, we extracted Gaussian parameters that allowed us to determine our threshold values. Specifically, we found the areas where GSR contains a high concentration of Cu and Pb and created two threshold values that separate GSR samples from non-GSR samples. This method was more suitable to real field-collected GSR samples acquired during field trips.

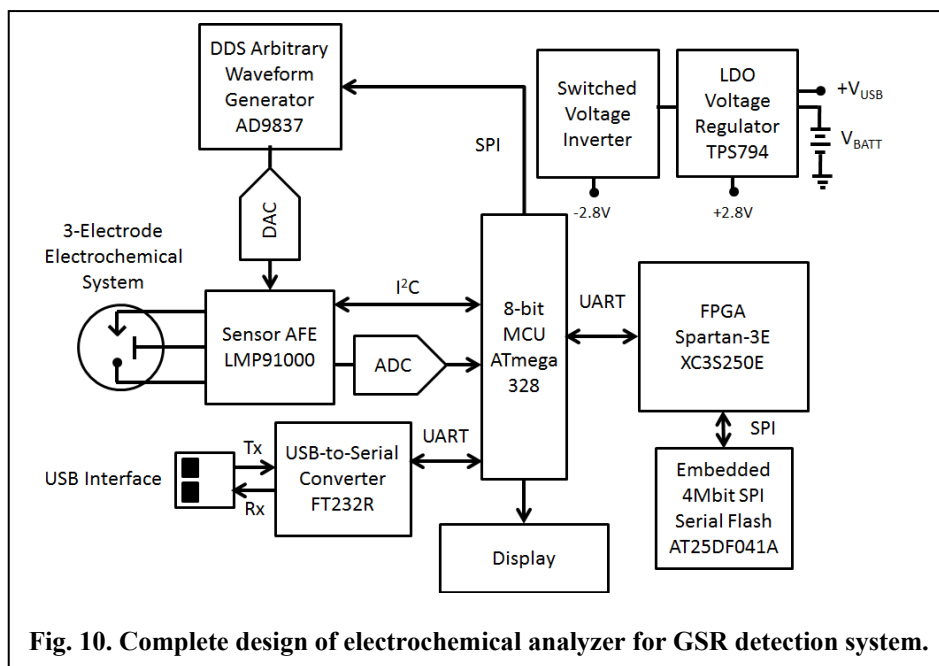
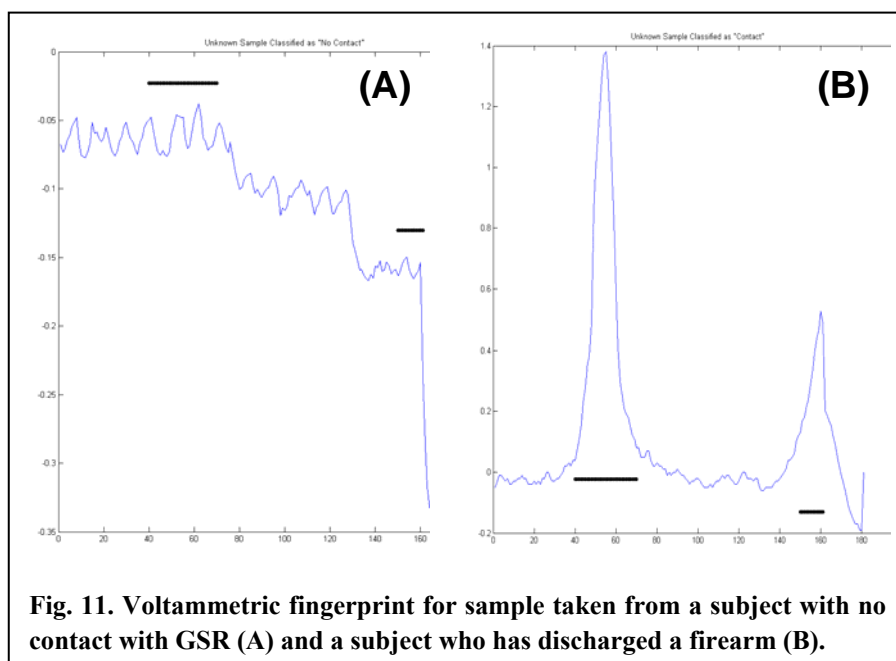


Fig. 10. Complete design of electrochemical analyzer for GSR detection system.

The model shown in Fig. 9 was generated utilizing 36 samples taken from a trip to the shooting range and gave an accuracy of 100% for training. It was then validated utilizing 48 samples taken from a second trip to the shooting range, and gave an accuracy of 98%. Therefore this analysis tool was brought forward to the next phase of this project for the development of a prototype containing all required electronics, power and analysis.

d. Task 4: Design of hand-held analyzer

Our goal for this task was to emulate a conventional analytical laboratory within a handheld device by leveraging prior expertise in blood glucometers and metalyzers. Constraints



shown in Fig. 10.

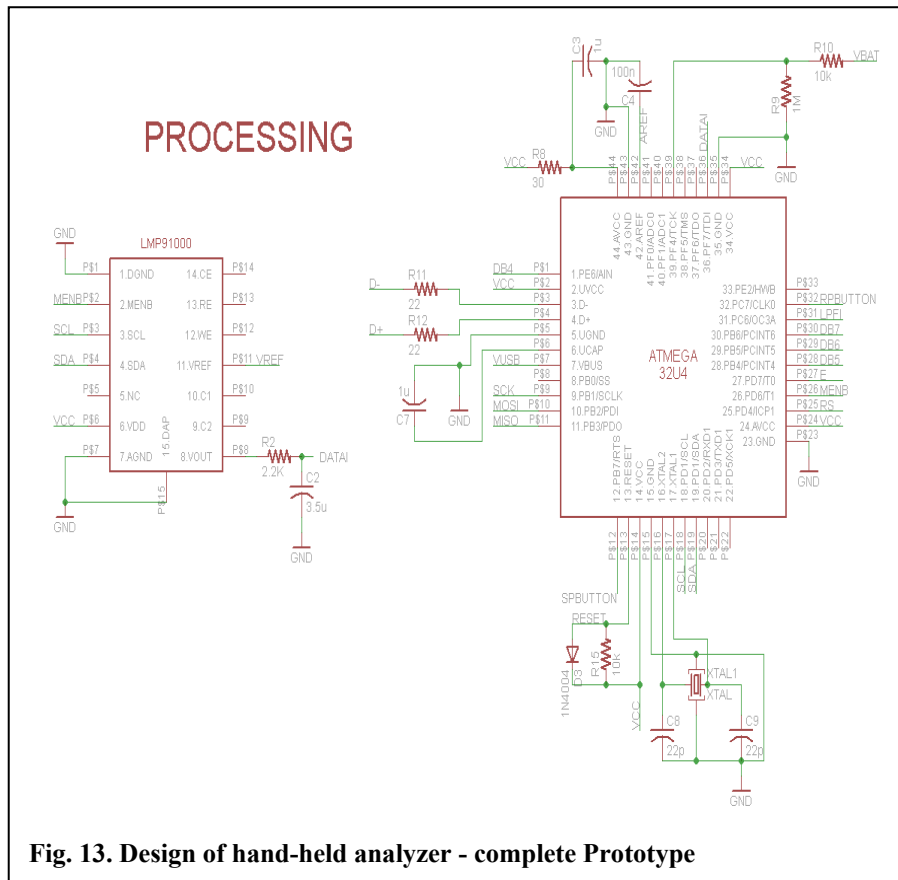
This system contained the required tools to complete analysis for ‘Involved’ or ‘Not Involved’. The difference in actual voltammetric readouts from the bread boarded system for GSR samples comparing ‘Not Involved’ and ‘Involved’ are shown in Fig. 11 A and B respectively. The voltammetry in Fig. 11A showed a distinct lack of voltammetric signals whereby there was no fingerprint to meet the threshold levels for presence of GSR. Therefore, we were given readout of ‘No GSR’. However, in Fig. 11B, for a sample taken from a subject who has discharged a firearm, the signals were significantly above the threshold levels designated, and the analyzer gave a readout of ‘GSR Detected’. We utilized this breadboard system for ‘synthetic’ GSR samples – mixtures of Pb, Sb and Cu. Under the existing parameters, we obtained a LOD >1ppm. We therefore optimized the parameters implemented, such as TIA settings, low pass filters (20 Hz is optimal), accounted for LPF delays and increased deposition time (300 s optimal). We then tested field samples which provided discernible differences between Load & Fire compared to other control scenarios

Utilizing these new parameters, new field samples were taken and Gaussian parameters were extracted to build a

encountered included mating of the potentiostatic control with advanced embedded signal processing algorithms (<5min from swipe to result) to generate a simple-to-operate, field-deployable unit offering a simple readout. The complete prototype was designed using CAD to contain the electronics, power system and signal processing. This is



Fig. 12. Electronic readout for GSR sample on bread boarded GSR screening tool.



detection model. Bayes decision rule was implemented on Arduino microcontroller and old field samples were run with determined decision rule parameters. The decision function tested further by scanning samples taken from used bullet cases which all displayed a reading of ‘GSR Detection’ (readout shown in Fig. 12).

We then tested this system with real GSR samples taken from the shooting range. Control scenarios included Not

Involved (N-No contact; S-Secondary; P-Presence) and Involved (L-Load; F-Fire; Per-Persistence). All together, 3 samples from 36 misclassified: 2x Persistence samples (45 min) and 1x Secondary sample, yielding a total of 92% accuracy when tested with in-field samples. We used these GSR samples to update threshold parameters and using new parameters, ran a new set of GSR field samples with the results of 2 samples from 36 misclassified: 1x Persistence sample (45 min) 1x Secondary sample yielding a total of 94% accuracy when tested with updated parameters.

Based on field tests, schematic and board layout for the device were finalized and sent to a manufacturer. Fig. 13 shows the design of the board. We received device prototype boards however faced many challenges with the following: LEDs, linear regulator and boost convertor. The LEDs are used to indicate when the board is connected to a computer and being charged and the manufacturer error has been rectified. The linear regulator should take in 3.7V and output a stable 3.3V, but we discovered that the chips “Bypass” pin should not have been grounded. We removed the pin, regulator now works. Finally, the boost converter should take in 3.3V and output a stable 5V which it does not, but we were making progress with this error.

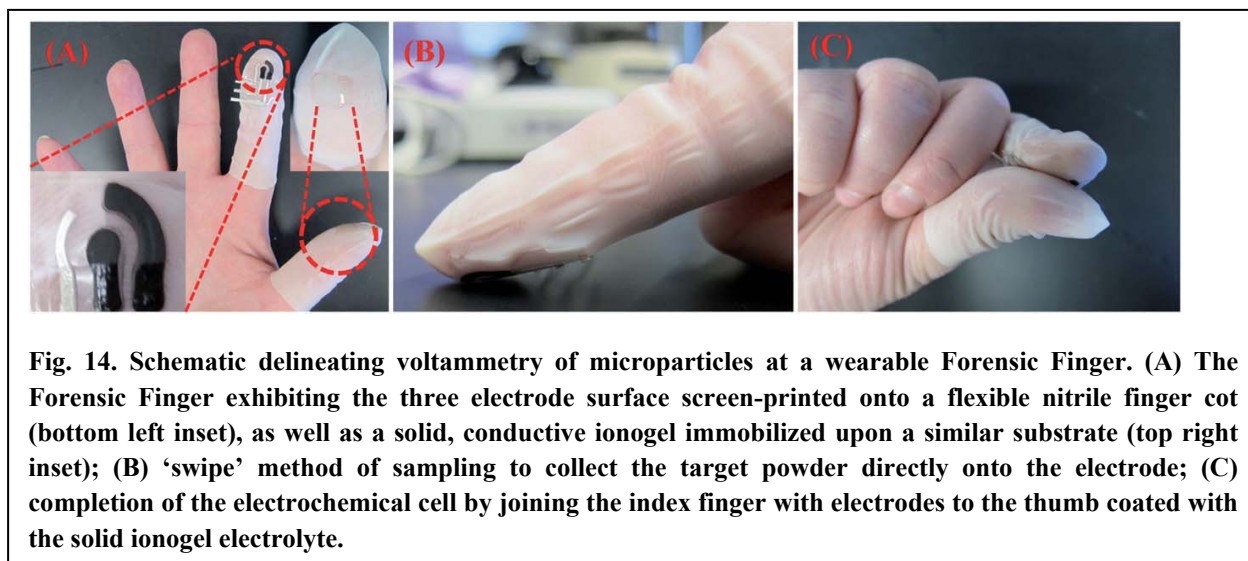
e. Task 5: : Critical testing and validation

Our goal for this task was to determine the analytical figures of merit under field conditions and demonstrate >97% accuracy in firing tests utilizing the prototype device received from the manufacturer. Constraints included several issues encountered with the LEDs, linear regulator and boost converters. The LEDs and linear regulator difficulties were addressed and rectified. We suggest that the boost convertor is the next challenge addressed so that future work can proceed for the testing of this system at the gun range utilizing real samples under a variety of different scenarios. We anticipate a high level of accuracy with this device based on previous tests examined at the bread board device. We also suggest a simple optimization of experimental parameters such as deposition potential and deposition time to decrease the 5 min period over which samples are deposited and analyzed.

f. Further Innovations

We also developed two further innovations as part of this project that is outlined below.

The first innovation involved a new detection system called the ‘Forensic Finger’. This work presents a simple, all-solid-state, wearable fingertip sensor for the rapid on-site voltammetric screening of GSR and explosive surface residues. To fabricate the new Forensic Fingers, we screen-printed a three electrode setup onto a nitrile finger cot, and coated another finger cot with an ionogel electrolyte layer. The new integrated sampling/detection methodology relied on ‘voltammetry of microparticles’ (VMP) and involved an initial mechanical transfer of trace amounts of surface-confined analytes directly onto the fingertip-based electrode contingent. Voltammetric measurements of the sample residues were carried out upon bringing the working electrode (printed on the index finger cot) in direct contact with a second finger cot coated with an ionogel electrolyte (worn on the thumb), thus completing the solid state electrochemical cell. Sampling and screening were performed in less than four minutes and generated distinct voltammetric fingerprints which were specific to both GSR and explosives. The use of the solid, flexible ionogel electrolyte eliminated any liquid handling which resolved problems associated with leakage, portability and contamination. A detailed study revealed that the fingertip detection system rapidly identified residues of GSR and nitro aromatic compounds with high specificity, without compromising its attractive behavior even after undergoing repeated mechanical stress. This new integrated sampling/detection fingertip strategy holds considerable promise as a rapid, effective and low-cost approach for on-site crime scene investigations in various forensic scenarios. Such effort has resulted in the publication of ‘Solid-state Forensic Finger sensor for integrated sampling and detection of gunshot residue and explosives: towards ‘Lab-on-a-finger’’. *Analyst* **2013**, 138, 5288



Following the fabrication of the Forensic Finger, the user adorned the electrode-printed finger cot on the index finger and the ionogel-modified finger cot on the thumb (Fig. 14A). To investigate a surface for possible GSR/explosives residues, the user gently abraded the index finger – containing the finger cot with the printed electrodes – on the surface (Fig. 14B) and then brings it in contact with the ionogel-coated thumb to complete the electrochemical cell (Fig. 14C). The sample was then analyzed using rapid square wave voltammetry with a field-portable electrochemical analyzer. The complete process was carried out independently by the user within four minutes.

Utilizing SWSV, we observed a distinct pattern corresponding to traces of GSR from the surface of ammunition. Fig. 15A outlines a voltammogram for the ionogel in the absence of GSR (black) as well as the voltammetric fingerprint recorded subsequently to swiping a GSR-rich surface (red). The voltammetric pattern for this GSR sweep was very distinct and was shown to be characteristic from scan to scan. We observed three voltammetric signals at potentials -0.6 V, -0.4 V and -0.2 V, which were attributed to Pb, Sb, and Cu, respectively. Previous data

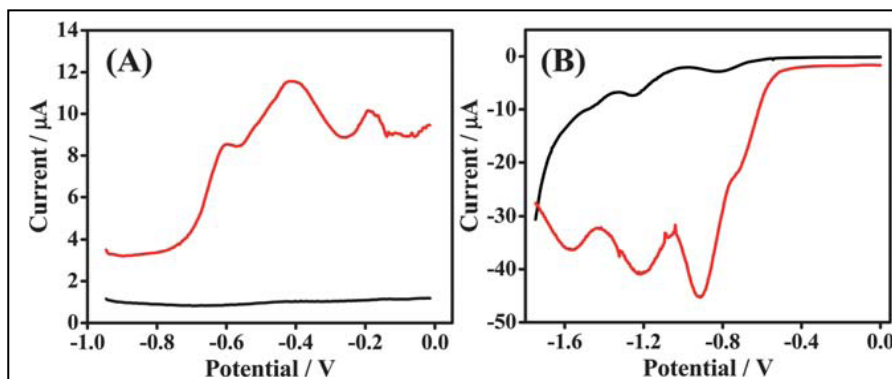


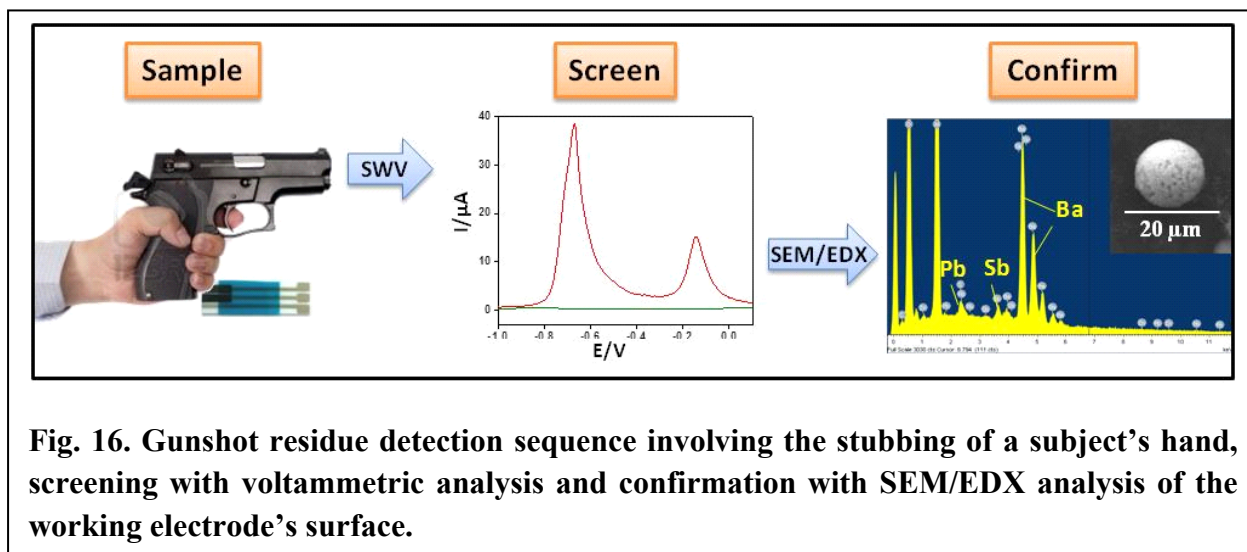
Fig. 15. Voltammetric response obtained at Forensic Finger sensor/ionogel interface in the absence (black) and in the presence (red) of (A) GSR & (B) DNT.

demonstrated that these metals strip at similar potentials on a similar electrode surface. We also attained the voltammetric signature of DNT by scanning reductively upon swiping the Forensic Finger over a DNT-rich surface. Fig. 15B displays voltammograms for the reductive scan of the

ionogel in the absence (black) and in the presence (red) of DNT. The electrochemical fingerprint of this nitro aromatic compound was very distinct compared with that of the blank ionogel scan due to the presence of easily-reducible nitro groups. Three signals were observed for this reductive scan at potentials -0.9 V, -1.2 V and -1.6 V. The first two signals at -0.9 V and -1.2 V were attributed to the stepwise reduction of the two nitro groups of DNT to hydroxylamine groups, while the third signal (at -1.6 V) was attributed to the reduction of one of the hydroxylamine groups to an amine.

In this work we have demonstrated that the ionogel electrolyte is stable over a week-long period. We demonstrated the robustness of the three electrode Forensic Finger sensor through mechanical stress studies and illustrated that the characteristic voltammetry of both GSR and DNT was retained. The integrated sampling and analysis steps, along with the removal of liquid handling and rapid square-wave voltammetry, ensured results within a four minute time frame. We demonstrated the practical application of this fingertip sampling/detection system by presenting the distinct voltammetric response for GSR in a 'GSR-rich' environment as well as the voltammetric fingerprint of GSR immobilized from the hand of a subject subsequent to the handling of a firearm. The new concept holds considerable promise as a portable, field-deployable screening method aimed at the rapid identification of a security threat or providing forensic evidence from either firearms or explosives.

A further innovation attributed to this funding was that of the orthogonal detection of GSR utilizing voltammetry, SEM and EDX analysis. This protocol implemented the orthogonal identification of the presence of GSR utilizing square-wave stripping voltammetry (SWSV) as a rapid screening tool along with scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX) to confirm the presence of characteristic morphology and metal composition of GSR particles. This was achieved through the judicious modification of the working electrode of a carbon screen printed electrode (CSPE) with carbon tape (used in SEM analysis) to fix and retain a sample. A comparison between a subject who has handled and loaded a firearm and a subject who has had no contact with GSR showed the significant variations in voltammetric signals and the presence or absence of GSR-consistent particles and constituent metals. This initial electrochemical screening had no effect on the integrity of the metallic particles, and SEM/EDX analysis conducted prior to, and post voltammetry showed no differences in analytical output. The carbon tape was instrumental in retaining the GSR sample after electrochemical analysis, supported by comparison with orthogonal detection at a bare CSPE. This protocol shows great promise as a two-tier detection system for the presence of GSR from the hands of a subject, whereby initial screening can be conducted rapidly, onsite by minimally trained operators, and confirmation can follow at the same substrate to substantiate the voltammetric results. Such efforts resulted in the submission of 'Orthogonal identification of gunshot residue with complimentary detection principles of voltammetry, scanning electron microscopy and energy-dispersive x-ray spectroscopy: Sample, Screen & Confirm' for publication in *Analytical Chemistry*.



The sequence of integrated sampling and analysis for screening, and orthogonal detection methods is outlined in Fig. 16. Sampling was carried out using a variation of the 'swipe and scan' method outlined in previous literature. The working electrode surface of the modified screen printed carbon electrode was deployed over the hands of a suspect using a stubbing mode of sample collection, allowing the SEM tape electrode contingent to stick to the thumb and back of the subjects' hand. The sample was then immediately ready for electrochemical analysis. SWSV was employed to identify the electrochemical targets of Pb, Sb and Cu. A distinct increase in signals for the presence of GSR (red) compared to the absence (green) was observed in the voltammetry of Fig. 16. Due to the presence of the carbon tape, the sample remained fixed to the working electrode surface subsequent to electrochemical detection. The sample was then loaded into the SEM, without any further modifications, and the GSR sample remained intact on the working electrode surface. SEM equipped with EDX provided close examination of particle morphology and high sensitivity analytical information of individual particles. The GSR particles displayed distinctive morphology of a spherical, 'cracked shell' appearance. Subsequent EDX analysis identified the presence of Ba, Sb and Pb, all three together being unique to GSR. The minimum emission voltage values of each metal are 4.465 (L α), 3.604(L α), 2.342 (M) keV for Ba, Sb and Pb respectively resulting in no overlap peaks or error of EDX analysis.

We demonstrated the orthogonal detection of GSR using the screening tool of SWSV, and the confirmation tools of SEM and EDX using samples taken from subjects in two different control scenarios. Fig. 17 outlines the analysis of a sample taken at a carbon tape-modified electrode from the hand of a subject who has loaded and handled a firearm (A), and a subject who has had no contact with GSR (B). For each of these control scenarios, SWSV was first performed (i) as the screening step, followed by analysis utilizing SEM (ii) and EDX (iii) as confirmation steps. Fig. 17 (A) shows the analysis for a subject who has loaded and handled a firearm at the carbon tape modified CSPE. The voltammetry in (i) was similar to previous reports and characteristic of the GSR voltammetric fingerprint, encompassing signals for Pb and Cu (with contributions from Sb) at potentials -0.7 V and -0.15 V, respectively. Upon completion of voltammetric screening, the electrode was placed into the SEM and images were taken of GSR particles. Fig. 17A (ii) shows a cracked-shell spherical morphology indicative of GSR, which confirmed the presence of GSR on the hand of the subject. Finally, EDX analysis was implemented on the working electrode surface. Fig. 17A (iii) shows the results of the dispersive analysis on the area over the particle shown in (ii). Clear signals were observed at values 2.342, 3.604 and 4.465 keV, corresponding to Pb, Sb, and Ba, respectively, which were noted to be

unique to GSR, thus confirming the presence of GSR particles on the hands of a subject.

Similar analysis was conducted on a sample taken from the hands of a subject who has had no prior contact with GSR. The results are outlined in Fig. 17B (i-iii). Fig. 17B (i) shows the voltammetry resulting from the scan of the 'No Contact' sample in the same potential window and the same current range as the scan taken in Fig. 17A (i). This scan displayed a featureless baseline for the voltammetric screening, and thus the subject was labeled as not involved.

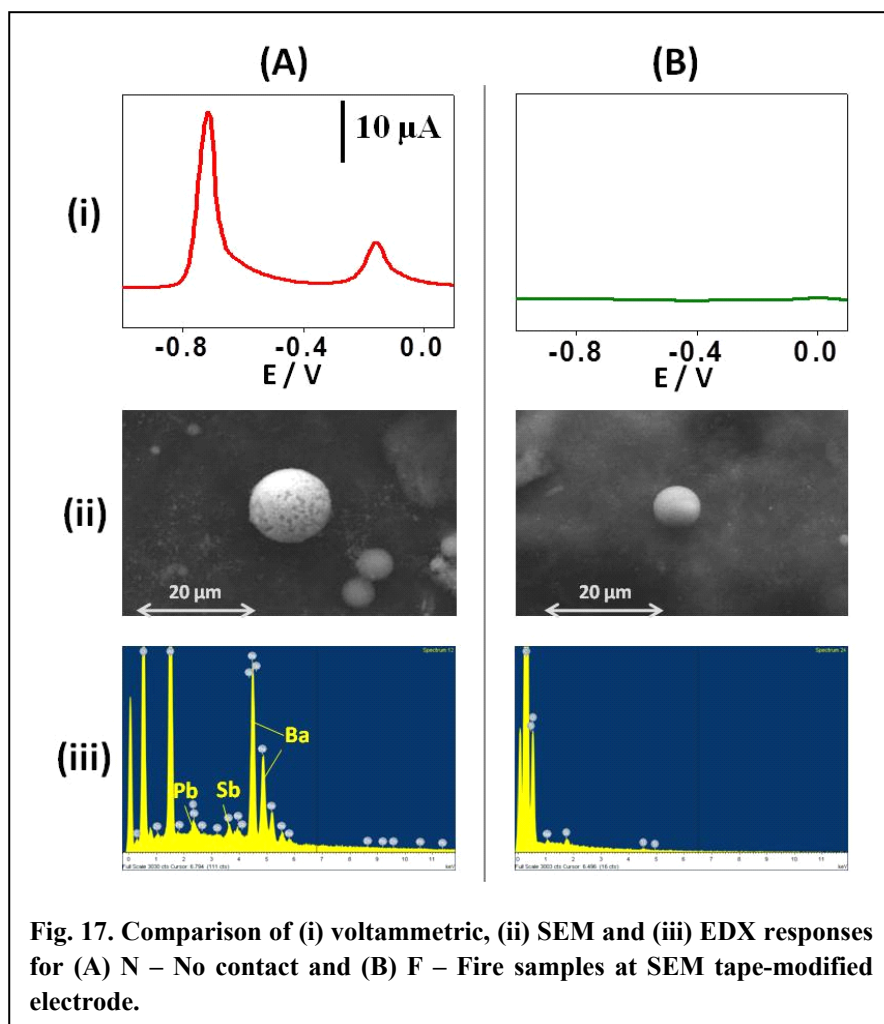


Fig. 17. Comparison of (i) voltammetric, (ii) SEM and (iii) EDX responses for (A) N – No contact and (B) F – Fire samples at SEM tape-modified electrode.

No particles fitting the description of the cracked-shell spherical morphology were observed during SEM analysis. Some spherical particles were observed, although these were smaller than the particles identified in Fig. 17A (ii). Upon inspection with EDX analysis, confirmation of absence of GSR particles was made, as outlined in Fig. 17B (iii). It is clear, when compared with the EDX scan from the subject in Fig. 17A (iii), a featureless scan to corroborate the SEM analysis and GSR screening was acquired. Thus no false positive identifications of a shooter were encountered.

The rapidity and portability of the initial voltammetric screening approach coupled with the specificity of the subsequent SEM/EDX analysis indicates promise for use of field-deployable, hand-held device for investigating firearm-related crimes, the substrate of which can later be further analyzed in a centralized laboratory.

Conclusion/Summary

The key accomplishments outlined in the above work include the successful determination of three metallic species in GSR in a single voltammetric run with 5 min. assay time. This represents the first example of using the same method for measuring simultaneously organic and inorganic GSR constituents. Our study illustrated that a mixture of these heavy metals and common propellants led to a distinct electrochemical signature, particularly in connection to cyclic square wave voltammetry. Such a unique, information-rich, single-run metal/propellant fingerprint provided a complete pattern of questioned and known signatures to determine if the constituents of the residue detected match those of suspects. We implemented the detection of all the species outlined in this work in a single, broad-potential scan at sensor-strip electrodes, utilizing both anodic and cathodic signals, to provide a portable GSR detection system.

As well as this we have successfully developed a facile, rapid, field-deployable method of sampling and analysis of GSR, nicknamed 'Swipe & Scan'. This new AbrSV protocol was based on an initial mechanical transfer of trace amounts of the immobilized GSR from the hand of the suspect directly to the working electrode surface of the sensor strip obviating the need for intermediate processing steps. The use of this fast and inexpensive protocol has been found to be effective for immediate analysis using ASV.

We also coupled discriminant functional analysis as well as Bayesian analysis with electrochemical signals towards effective discrimination of GSR samples from relevant scenarios as well as minimal false alarms with control samples. Effective discrimination of the level of contact with GSR for different subjects examined in this study was achieved using a variety of control experiments relevant to various forensic scenarios. Simplification of the controls involving no contact with the firearm improved the applicability of this system in real-world cases (screening of 'Involved' vs. 'Not Involved') as a screening tool for minimally trained operatives in the field.

The development of a prototype containing all required electronics, power and analysis was bread-boarded and data processing parameters were optimized for previously-collected in-field voltammetric signatures. Utilizing these parameters blind field-test samples were acquired and tested achieving an overall accuracy of 94%. Implementation of sampling and analysis from breadboard electronics to handheld prototype has commenced. We have received the complete prototype from the manufacturer and have addressed initial issues encountered with the LEDs and the linear regulator. We suggest that the boost convertor is the next challenge addressed so that future work can proceed for the testing of this system at the gun range utilizing real samples under a variety of different scenarios. We also suggest a simple optimization of experimental parameters such as deposition potential and deposition time to decrease the 5 min period over which samples are deposited and analyzed.

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